Atmospheric Effects on Plant Reproduction

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ATMOSPHERIC EFFECTS ON PLANT REPRODUCTION

FINAL REPORT

COOPERATIVE AGREEMENT NO. 28-CO-527

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WASHINGTON, D.C.

9/92

INTRODUCTION

The effect of acid rain on plants has generated considerable interest within the scientific and political community (Defries and Malone,1989; Hutchinson and Meema, 1987; Linthurst, 1984; Treshow, 1984). Despite this interest, relatively little is known about the effects of acid rain in natural plant communities, since most studies have focused on injury to vegetative tissues in economically important species (Evans et al, 1981; Lee et al, 1981). Our experiments examine the effects of acid rain on vegetative and reproductive processes in natural populations of alpine plants at the Glacier Lakes Ecosystems Experiments Site (GLEES) within the Medicine Bow National Forest.

Previous investigations at this site have demonstrated reduced pollen germination and growth in response to low pH in a variety of herbaceous species (McKenna, 1990). Field pollination studies also revealed significantly reduced seed sets in *Aquilegia caerulea* plants exposed to simulated acid rain. Certain species may be more tolerant however; studies in *Erythronium grandiflorum* indicate less sensitivity to low pH during pollen germination and no significant reduction in seed sets of plants exposed to simulated acid rain.

A primary aim of the present study was to further investigate the reproductive effects of acid precipitation on *Aquilegia caerulea* and *Erythronium grandiflorum*, and to investigate the effect of acid rain on vegetative growth in *Aquilegia caerulea*. We also designed a series of laboratory and field experiments to investigate the effects of acid rain on reproduction in *Penstemon whippleanus* populations at different altitudes. Finally we carried out a group of laboratory germination studies to compare the pH sensitivity of pollen from four species of the genus *Pedicularis* that are abundant at GLEES.

MATERIALS AND METHODS

Effects of simulated acid rain on *Aquilegia caerulea*

I. Effect of pH on pollen germination in vitro

Pollen was freshly collected from thirty plants in the Meadow Creek population (the site of the 1989 study). We acidified the standard Brewbaker Kwack germination media (Brewbaker and Kwack, 1963) with a 1:1 solution of sulfuric and nitric acid to create six experimental germination medias of pH 2.5 to 7.5. Half of the trials were run with media containing 10% sucrose osmoticum and half were run with media containing 0.29 M polyethylene glycol(M.W = 400) osmoticum and 10^{-3} M Tris (Roberts et al, 1983). Seventy five microliters of media was added to depression slides containing 100-200 pollen grains. The slides were placed on saturated filter paper in a closed Petri dish to maintain high humidity. The slides were incubated in a lab oven for two hours at 26 degrees Centigrade. After incubation, the pollen was stained for viability using Alexander's stain (Alexander, 1969), and % germination, % viability and total pollen grain number were determined by counting five random fields at 100x magnification. Tube lengths were measured from central grains in each of the five fields using an ocular micrometer, and an average was obtained for each sample. Four additional samples

per pH value were included in each assay in order to measure the pH of media with and without pollen at the conclusion of the two hour incubation period.

II. Acid rain simulant effect on seed set

150 buds were destaminated on 39 plants in the Meadow Creek population that had not been previously treated (3-6 flowers per plant). All flowers were bagged with cotton mesh pollinator exclusion bags. Plants were marked with permanent numbered pin flags. Six days later the styles had matured (started to recurve). Bags were removed and flowers were sprayed with acid rain simulant (pH 3.5) or ambient rain simulant (pH 5.6), 4 ml (5 sprays) per flower. When possible, the flowers on each plant were divided so that half received an acid spray treatment and half received an ambient spray treatment. Rain simulant solutions were prepared using the background ion concentrations measured by NADP collections at the GLEES site. The solutions were prepared and pH adjusted using the methods described for previous studies (McKenna, 1990). Flowers were tagged with gummed labels indicating the spray treatment and covered again with the cotton pollinator exclusion bags.

A total of 131 flowers on thirty two plants were pollinated using pollen from 19 pollen donor plants in the Meadow Creek population. The pollinations were carried out over three days. Thirty ambient-spray flowers and twenty eight acid-spray flowers were pollinated 24 hours after spraying, twelve ambient and twelve acid flowers were pollinated 48 hours after spraying, and an additional 15 ambient treatment flowers and 13 acid treatment flowers were pollinated 72 hours after spraying. Half of the flowers in each treatment were pollinated with pollen donors from the marked population, and the other half were from additional unmarked plants in the Meadow Creek population. When possible, flowers from both spray treatments on each plant received pollen from the same pollen donor. Pollen was applied by evenly coating all stigma tips with abundant pollen from anthers held in forceps. Flowers were bagged again following pollination. Fruits were collected 30 days after pollination, placed in individual coin envelopes, and seed set per fruit was determined.

III. Acid rain simulant effect on vegetative growth

Newly emerging plants were divided into three groups: one group received a misting of 350 ml ambient rain simulant (pH 5.6) every other day for seven weeks, the second group received a misting of 350 ml acid rain simulant (pH 3.5) every other day for seven weeks, and the third group was a non-spray control group. The heights of five individually marked stems were measured on each plant at approximately 10 day intervals, and total plant height and crown diameter was also measured on each plant. Soil samples at 3 cm depth were collected underneath the plants at three intervals throughout the study for measurement of soil pH. We also counted the numbers of flowers and fruits produced by plants in all three groups. Pollen from flowers produced by plants in the ambient-spray group and the acid-spray group was also collected to compare pollen germination in vitro at pH 7.5, 6.5, 5.5, 4.5, 3.5 and 2.5. The techniques used for this study are the same as those outlined in Section I above. At the conclusion of the seven week study, preliminary data was obtained to compare stomatal conductance of plants in the three experimental groups using a LiCor steady state porometer. Leaves from all three groups were collected for SEM analysis of surface damage or cuticular

erosion resulting from the spray treatments.

Additional acid and ambient rain spray treatments were carried out on these plants during summer 1991. Sixteen plants per treatment (including all twelve plants per treatment from the 1990 study) were marked with pin flags, and five stems per plant were individually labelled. Each stem received 12 ml (15 sprays) of rain simulant on 8 treatment dates over a three week period (7/10/91 - 7/31/92). At the conclusion of this experiment, leaves from all treated stems and from stems on 16 additional non-sprayed plants were collected and fixed in ethanol for analysis of leaf damage using a scanning electron microscope.

Effects of simulated acid rain on *Erythronium grandiflorum*

I. Effect of pH on pollen germination in vitro

Pollen was freshly collected from plants at the Meadow Creek field site. We acidified the standard Brewbaker Kwack germination media (Brewbaker and Kwack, 1963) with a 1:1 solution of sulfuric and nitric acid to create six experimental germination medias of pH 2.5 to 7.5. Half of the trials were run with media containing 10% sucrose osmoticum and half were run with media containing 0.29 M polyethylene glycol (M.W = 400) osmoticum and 10^{-3} M Tris (Roberts et al, 1983). Seventy five microliters of media was added to depression slides containing 100-200 pollen grains. The slides were placed on saturated filter paper in a closed Petri dish to maintain high humidity. The slides were incubated in a lab oven for two hours at 26 degrees Centigrade. After incubation, the pollen was stained for viability using Alexander's stain (Alexander, 1969), and % germination, % viability and total pollen grain number were determined by counting five random fields at 100x magnification. Tube lengths were measured from central grains in each of the five fields using an ocular micrometer, and an average was obtained for each sample. Four additional samples per pH value were included in each assay in order to measure the pH of media with and without pollen at the conclusion of the two hour incubation period.

II. Acid rain simulant effect on seed set

One hundred *Erythronium* buds in the Meadow Creek population were destaminated and covered with cotton mesh pollinator exclusion bags. Three days later, after all destaminated buds had opened and the styles were exserted, half of the flowers were sprayed with 4 ml (5 sprays) of acid rain simulant (pH 3.5), and half the flowers were sprayed with 4 ml (5 sprays) of ambient rain simulant (pH 5.6). Flowers were tagged and rebagged following the spray treatment. Twenty four hours after the spray treatment, flowers were pollinated by covering the tip of the style with abundant pollen from an anther held in forceps. Pollen was collected from flowers in the Meadow Creek population that had at least one remaining unopen anther, to assure that all donor flowers were approximately the same age. Each pollen donor was used to pollinate at least one acid-sprayed flower and one ambient-sprayed flower. Pollinator exclusion bags were replaced on all pollinated flowers. Fruits were collected and placed in individual coin envelopes; seeds from all fruits were counted.

III. *Erythronium grandiflorum* soil microhabitat study

Five soil samples were collected with a spade from four locations within the Meadow Creek population site. Location 1 was a collection site 2 inches below an intact snowpack. At the time of collection the tips of *Erythronium* buds could be seen emerging from the snowpack. Location 2 was a collection site at the surface of the intact snowpack. Location 3 was a collection site at the surface of the melting snowpack perimeter. At the time of collection *Erythronium* plants could be seen fully emerged, but without open flowers in this location. Location 4 was a collection site at the surface 4 inches from the perimeter of the melting snowpack. At the time of collection *Erythronium* plants with open flowers could be seen at this location.

Soil samples were transported to the lab in ziploc plastic bags, and allowed to air dry for five days. Soil pH was measured by suspending one gram of soil sample in 100 ml of distilled water and measuring the pH of the soil solution while stirring. All pH measurements were made with a Fisher Accumet pH meter standardized at pH 7.0 and pH 4.0.

Effects of simulated acid rain on *Penstemon whippleanus*

I. Effect of pH on pollen germination in vitro: Comparison of three populations at different altitudes

Pollen was freshly collected from plants in three populations: Meadow Creek (altitude 3340 m), Mountain Meadow (altitude 3000 m) and Centennial Lodge (altitude 2500 m). We used a Brewbaker Kwack germination media (Brewbaker and Kwack, 1963) with a 0.29 M polyethylene glycol (M.W = 400) osmoticum that was acidified with a 1:1 solution of sulfuric and nitric acid to create six experimental germination medias of pH 2.5 to 7.5. Fifty microliters of media was added to depression slides containing 100-200 pollen grains. The slides were placed on saturated filter paper in a closed Petri dish to maintain high humidity. The slides were incubated in a lab oven for two hours at 28 degrees Centigrade. After incubation, the pollen was stained for viability using Alexander's stain (Alexander, 1969), and % germination, % viability and total pollen grain number were determined by counting five random fields at 100x magnification. Tube lengths were measured from central grains in each of the five fields using an ocular micrometer, and an average was obtained for each sample. Four additional samples per pH value were included in each assay in order to measure the pH of media with and without pollen at the conclusion of the two hour incubation period.

II. Acid rain simulant effect on seed set

We carried out a pollination study at the middle elevation site (Mountain Meadow) that was designed to investigate the effect of simulated acid rain on fruit development and seed set in a natural population of *Penstemon whippleanus*. One hundred and fifty buds on thirty plants were destaminated and covered with cotton mesh pollinator exclusion bags. Four days later, 125 flowers were sprayed with 4 ml (5 sprays) of acid rain simulant (pH 3.5) or ambient rain simulant (pH 5.6). Flowers at the same node of each plant received the same spray treatment to avoid cross contamination. When possible each plant contained flowers that had

received acid spray and flowers that had received ambient spray. The node position (top, middle, bottom) was noted for all experimental flowers, and equalized for both spray treatments. Pollinator exclusion bags were replaced on all flowers immediately after spraying. Pollinations were carried out twenty four hours after spraying using pollen donors selected from within the Mountain Meadow population. Pollination was achieved by covering the tip of the style with abundant pollen from an anther held in forceps. When possible, each pollen donor was used to pollinate acid-sprayed flowers and ambient sprayed flowers. Pollinator exclusion bags were replaced on all flowers immediately after pollination. Fruits were collected 36 to 42 days following pollination, placed in individual envelopes, and seed counts of all fruits were made.

III. Acid rain simulant effect on stigma pH

A study was designed to measure the pH of *Penstemon whippleanus* stigmas after treatment with acid or ambient rain simulant sprays, and with or without pollen. Flowers from the mid-elevation (Mountain Meadow) site were used for this experiment. Two hundred flowers on thirty three plants were destaminated and covered with cotton mesh pollinator exclusion bags. Two days later 70 flowers were sprayed with 4 ml (5 sprays) of acid rain simulant (pH 3.5) and 70 flowers were sprayed with 4 ml (5 sprays) of ambient rain simulant (pH 5.6). The remaining destaminated flowers were left unsprayed. Flowers at the same node of each plant received the same spray treatment to avoid cross contamination. When possible each plant contained flowers that had received acid spray, flowers that had received ambient spray and flowers that had not been sprayed. The node position (top, middle, bottom) was noted for all experimental flowers, and equalized for both spray treatments. Pollinator exclusion bags were replaced on all flowers immediately after spraying. Half of the flowers in each treatment category (acid-spray, ambient-spray, non-spray) were pollinated twenty four hours later using pollen donors from the Mountain Meadow population. Pollination was achieved by covering the tip of the style with abundant pollen from an anther held in forceps. When possible, each pollen donor was used to pollinate acid-sprayed flowers, ambient sprayed flowers and non-sprayed flowers.

Pollinator exclusion bags were replaced on all flowers immediately after pollination. Pistils of all flowers were collected two hours after the pollinations were complete. The stylar tip of each pistil was immersed in distilled water in an conical plastic microcentrifuge tube and allowed to remain undisturbed for twenty four hours. After this time, the styles were removed and the pH of the immersion solution was measured using a Fisher Accumet pH meter standardized at pH 7.0 and pH 4.0.

IV. Variation in floral morphology in two populations at different altitudes

Measurements of floral morphology were made in flowers collected from the Meadow Creek alpine field site and from the Mountain Meadow mid-elevation field site. Measurements were taken of the length of the upper corolla and the width of the corolla opening. Node positions were also recorded on all flowers measured.

Effects of simulated acid rain on *Pedicularis spp.*

I. Effect of pH on pollen germination in vitro: Comparison of three species of *Pedicularis*

Pollen was freshly collected from plants at elevations ranging from 3200 meters to 3300 meters throughout the GLEES site. We acidified the standard Brewbaker Kwack germination media (Brewbaker and Kwack, 1963) with a 1:1 solution of sulfuric and nitric acid to create six experimental germination medias of pH 2.5 to 7.5. Trials were run with media containing 10% sucrose osmoticum and with media containing 0.29 M polyethylene glycol (M.W = 400) osmoticum and 10^{-3} M Tris (Roberts et al, 1983). Fifty microliters of media was added to depression slides containing 100-200 pollen grains. The slides were placed on saturated filter paper in a closed Petri dish to maintain high humidity. The slides were incubated in a lab oven for two hours at 26 degrees Centigrade. After incubation, the pollen was stained for viability using Alexander's stain (Alexander, 1969), and % germination, % viability and total pollen grain number were determined by counting five random fields at 100x magnification. Tube lengths were measured from central grains in each of the five fields using an ocular micrometer, and an average was obtained for each sample. Four additional samples per pH value were included in each assay in order to measure the pH of media with and without pollen at the conclusion of the two hour incubation period.

RESULTS

Aquilegia pollen demonstrated high viability (82% -94%) during the germination tests in vitro, and showed a significant decrease in germination percentage below pH 4.5 in both medias (Table 1). The decrease in germination was most extreme in the Brewbaker/Kwack - sucrose media (BK-S), where a sharp decrease was observed below pH 5.5. Average pollen tube length also decreased below pH 4.5. These results are similar to the results obtained with this species in earlier studies (McKenna, 1990). Germination and tube length also decreased at pH above 6.5, suggesting that a pH range of 5.5 to 6.5 may be optimal for *Aquilegia* pollen.

Pollen collected from plants that had been sprayed with acid rain simulant or ambient rain simulant (Table 2) demonstrated a similar inhibition of germination below pH 4.5. Pollen viability was greater for pollen obtained from plants that had been treated with ambient rain simulant (mean = 90%; range = 86% - 93%) than from plants treated with acid rain simulant (mean = 83%; 71% - 90%).

The *Aquilegia* fruits resulting from hand pollinations following acid and ambient spray treatments did not fully mature in the interval between pollination and collection. As a consequence, no fully mature seeds were obtained, but counts were made of all enlarged (maturing) seeds and all non-developed seeds (presumed to be unfertilized ovules or aborted seeds) in each fruit. The percentage of pollinated flowers that were harvested was high for both treatments, and approximately 80% of fruits harvested from both treatments were fertile (Table 3). There was significant variation in the number of ovules initiated by fertile flowers in both treatments, with values ranging from 4 to 313. Fruits resulting from the acid spray treatment had a significantly greater number of ovules than fruits resulting from the ambient

spray treatment (Table 3). This makes it difficult to directly compare the number of maturing seeds per fruit in the two treatments, since one expects greater seed set in fruits that have initiated more ovules. In this case, acid-sprayed flowers produced a greater number of ovules and a greater number of maturing seeds. A direct comparison can be made, however, between the proportion of ovules that resulted in maturing seeds in fruits resulting from the two experimental treatments. Flowers that were sprayed with acid rain simulant yielded fruits with a significantly lower proportion of maturing seeds than flowers that were sprayed with the ambient rain simulant (Table 3).

Table 4 shows the results of the stem height measurements obtained from *Aquilegia* plants in the acid-spray, ambient spray and non-spray treatments. At the start of the study there was no significant difference in the mean stem height of plants in the acid-spray or ambient-spray groups, but plants in the non-spray group were significantly taller than either of the other two groups. The non-spray plants maintained this significant difference in stem height at the end of the study. After the first measurement interval, the stem heights of plants receiving the acid spray treatment were significantly shorter than plants in the ambient spray treatment, and this difference persisted at all subsequent measurements. Total plant height was also reduced in the plants receiving the acid spray treatment (Table 5 and Figure 1). Crown diameter was also reduced in plants receiving the acid spray treatment (Table 6 and Figure 2). The non-spray plants produced nearly twice the number of flowers and fruits as the ambient-spray plants (Table 7); this probably reflects the fact that plants in the non-spray group were larger. Plants in the ambient spray group produced more flowers than plants receiving the acid spray treatment, but there was no difference in the number of fruit initiated by the date we concluded the study (Table 7). Soil pH values in the vicinity of the experimental plants did not vary much throughout the study period, and did not appear to be affected by the spraying treatments (Table 8).

Results from a pilot study of stomatal conductance and transpiration in *Aquilegia* plants from the three experimental groups is shown in Table 9. These data were recorded by students participating in the field course at GLEES sponsored by the Forest Service. The results suggest a higher rate of stomatal conductance in plants receiving both spray treatments compared to plants in the non-spray group, and a higher rate of stomatal conductance in plants receiving the acid spray treatment compared to plants in the ambient spray group. Transpiration rates appeared to be lower in plants in the non-spray group compared to plants in both spray treatment groups. Light levels varied greatly during measurements of the three experimental groups however. Dr. Robert Musselman is currently carrying out a more extensive study of leaf conductance and photosynthetic rate in *Aquilegia* plants receiving the same experimental treatments.

Differences in stomatal conductance could be related to leaf surface disruption through physical or chemical injury resulting from the spray treatment. Preliminary SEM analysis of leaf surfaces from plants in the three experimental groups suggests that significant disruption of the cuticle in acid-spray plants may have occurred (Figure 3). SEM studies comparing the extent of damage in leaves from all three experimental groups is still in progress.

Erythronium pollen demonstrated high viability (80% - 95%) during the germination tests in vitro (Table 10). Pollen from this species showed no sharp decrease in germination until it was exposed to media with pH below 3.5. These results are similar to results seen in previous studies with this species (McKenna, 1990). Average pollen tube length decreased from pH 5.5 to pH 2.5, however. Germination percentages remained high in medias ranging from pH 5.5 to pH 7.5.

The *Erythronium* fruits resulting from hand pollinations following acid and ambient spray treatments did not fully mature in the interval between pollination and collection. As a consequence, no fully mature seeds were obtained, but counts were made of all enlarged (maturing) seeds. No non-developed seeds were observed in these fruits. The percentage of pollinated flowers that were harvested was high for both treatments (Table 11). A larger proportion of fruits containing maturing seeds was observed in pollinations of acid-sprayed flowers compared to the ambient spray group, but fruits resulting from the acid spray treatment contained a significantly smaller number of maturing seeds than fruits resulting from the ambient spray treatment (Table 11).

Table 12 contains the results from the study of *Erythronium* soil microhabitats. There was little variation in soil pH observed at the four locations measured. Soil pH values ranged from 3.98 - 4.48. Mean pH measurements at each location ranged from 4.17 to 4.25.

When data from all three *Penstemon* populations was combined, *Penstemon* pollen demonstrated uniformly high viability (94% -97%) during the germination tests in vitro, and showed a significant decrease in germination percentage below pH 5.5, and a dramatic decrease in germination percentage below pH 4.5 (Table 13). Average pollen tube length also decreased in media below pH 5.5. Germination and tube length remained high in medias ranging from pH 5.5 to pH 8.5. Pollen from each of three populations demonstrated a similar response to pH levels, although the overall germination percentages for pollen from the lower elevation Centennial Lodge site were lower than the germination percentages observed in the other two populations.

The *Penstemon* fruits resulting from hand pollinations following acid and ambient spray treatments did not fully mature in the interval between pollination and collection. As a consequence, no fully mature seeds were obtained, but counts were made of all enlarged (maturing) seeds. No non-developed seeds or unfertilized ovules were observed in these fruits. The percentage of pollinated flowers that were harvested was high for both treatments (Table 14). In all experimental treatments a very small percentage of harvested fruits contained maturing seeds however. This may be due to the destamination procedure, since in most cases it was necessary to remove all or part of the corolla in order to remove the attached anthers. Removal of the corolla may have led to significant desiccation effects on the pistil. Among the small proportion of fruits that did contain maturing seeds, however, there were significantly fewer seeds in fruits resulting from the acid spray treatment compared to fruits resulting from the ambient spray treatment (Table 14).

Table 15 shows the results of our investigation of style pH variation in *Penstemon whippleanus*. We were able to detect very little variation in stylar pH following either spray

treatment and following the application of pollen. The measurements for samples under all experimental conditions ranged from pH 5.95 to pH 5.99. Little variation was observed in floral morphology in *Penstemon* flowers from the two populations measured (Table 16). Flowers from the high elevation Meadow Creek population were slightly longer and narrower than flowers from the middle elevation Mountain Meadow population. Flower morphology does not appear to be related to node position in *Penstemon whippleanus*.

A comparison of pollen germination and growth in vitro in three *Pedicularis* species exposed to media pH's ranging from pH 7.5 to pH 2.5 is shown in Table 17. The germination of pollen from all three species is significantly inhibited at media pH below 4.5, and sharp declines in germination are also observed in some cases below pH 5.5. Average pollen tube length is greatest in media pH's ranging from 5.5 to 7.5.

DISCUSSION

The results of our studies of *Aquilegia* pollen germination in vitro show a pattern of sensitivity below pH 4.5 that we have seen in our previous studies. Despite high viability and approximately equal numbers of pollen grains per well at all pH levels, we observed a clear inhibition of pollen tube germination and growth at media pH's below 4.5. In the media with sucrose osmoticum (BK-S), pollen germination sharply declined below pH 5.5, and pollen germination and growth appeared optimal at pH 5.5. Longer pollen tubes were observed in the BK-S media; this may reflect a nutritive role of sucrose in this media. The inclusion of a higher media pH in the present study (pH 7.5) allowed us to observe that germination and growth of *Aquilegia* pollen appears to be inhibited at high pH levels as well as at low pH levels. In pollen collected from plants sprayed with acid rain simulant throughout the growing season, there is no evidence of increased tolerance to low pH due to mortality of sensitive pollen genotypes during development. The acid spray treatment may have contributed to the decreased viability and the greater variation in viability of pollen from the plants sprayed with acid rain simulant, however.

The results of the *Aquilegia* pollination study also agree with our earlier studies demonstrating decreased reproductive ability in plants exposed to acid rain simulant. In the present study, since we observed a wide variation in ovule number per fruit, and all fruits were collected before reaching maturity, we calculated reproductive ability as the proportion of ovules that resulted in maturing seeds. A significantly smaller proportion of ovules matured from flowers sprayed with the acid rain simulant. This result suggests that fewer pollen tubes were able to successfully fertilize ovules in flowers sprayed with the acid rain simulant. Since we observe a significant decrease in *Aquilegia* pollen germination and growth in vitro at pH 3.5, it is likely that pollen growth inhibition may be responsible for the seed maturation pattern we observed. We found little evidence of seed abortion in fruits from this experiment, although seeds aborted very early are indistinguishable from unfertilized ovules. We are designing future studies to examine the pattern of variation in ovule number in *Aquilegia*. Studies by Brunet (1990) suggest that the first flowers initiated by *Aquilegia* may have the largest ovule numbers, and that female functional gender decreases with subsequent flowers.

Our studies demonstrate a clear effect of acid rain simulant on vegetative growth in *Aquilegia caerulea*. Total plant height, crown diameter and mean height of five random stems were significantly smaller in plants exposed to the acid rain simulant compared to the plants sprayed with the ambient rain simulant. The data also suggest the potential for decreased reproductive output in plants exposed to the acid rain simulant, since plants in this group initiated fewer flowers. We concluded the present study before all flowers had opened however, and there was no difference in fruit set between the two treatments up to this point. Future studies are being designed to examine pollination frequency and fruit set in plants exposed to acid or ambient rain simulant. The mechanism responsible for the growth reduction in plants exposed to acid rain simulant is not yet clear. Our soil pH measurements suggest that soil acidification during the period of spraying did not occur. Preliminary SEM analysis indicates severe disruption of the leaf cuticle in plants sprayed with acid simulant; this may be a result of physical injury or chemical processes. We are continuing to carry out a SEM analysis of samples from this study and the subsequent study (1991) that employed a smaller volume of rain simulant. These analyses will provide more insight into the anatomical basis of the observed differences in plant growth. Preliminary physiological studies also suggest that the spray treatments may have affected photosynthetic processes. Extensive physiological monitoring of plants sprayed with acid and ambient rain simulant, as well as unsprayed plants, is currently (Summer, 1992) underway to address these questions.

The results of the studies of *Erythronium grandiflorum* pollen germination and growth in vitro indicate that pollen from this species is more tolerant of low pH than pollen from *Aquilegia caerulea*. This result agrees with the data obtained from our previous studies of *Erythronium* (McKenna, 1990). In the current study, we obtained average pollen tube lengths at each media pH; tube lengths were successively shorter from pH 6.5 to pH 2.5. The decline in average pollen tube length was quite marked at pH 4.5 and pH 3.5, despite relatively good germination in media at these pH levels.

The pollination studies in *Erythronium grandiflorum* yielded unexpected and interesting results. There were significantly fewer seeds per fruit from flowers exposed to the acid rain simulant compared to the ambient rain simulant. These results differ from the results of a previous pollination study (McKenna, 1990) in which no difference in seed set between the two treatments was observed. In the previous study we pollinated flowers during a period 1-2 hours after spraying, while in the current study flowers were pollinated 24 hours after spraying. It is possible that in the earlier study, we pollinated flowers before allowing sufficient time for acid damage effects to be produced on stylar tissue. The data from this pollination study along with the data from the studies of pollen tube lengths in vitro suggests that reproductive processes in *Erythronium* may not be as tolerant to low pH levels as we first suspected. We have also obtained preliminary data from pollen studies in vivo (using pistils treated with uv-fluorescent dyes) that indicate a significant decrease in the number of pollen tubes in styles of flowers that were sprayed with acid rain simulant prior to hand pollination. We are currently designing and executing further studies on the effects of acid rain simulant on reproductive and vegetative processes in this species.

Very little variation was observed between populations of *Penstemon whippleanus* in terms of floral size and pollen response to media pH levels. Pollen of *Penstemon whippleanus*

demonstrated a sharp decline in pollen germination and tube length in medias below pH 4.5, and a more moderate decline between pH 5.5 and pH 4.5. The relative uniformity in pollen response between populations suggests that this response does not depend on specific habitat variables, but most likely represents a species-specific response. The low percentage of fruit set resulting from the hand pollination experiment may be due to the necessity of removing the corolla in order to remove the attached stamens. The exposed pistil may have experienced significant water loss or other physiological changes that resulted in poor germination and growth of pollen or inability to initiate and sustain fruit development. We are currently carrying out an expanded study of acid rain simulant effects in a population of *Penstemon whippleanus* (Summer, 1992). We have incorporated hand pollinations into this study, but designed the study so that it is unnecessary to destaminate the flowers prior to pollination.

Although pollen from all *Pedicularis* species examined demonstrated significant inhibition of germination and growth in media below pH 4.5, we observed variation between the species within this genus. *Pedicularis racemosa* appears to be the most tolerant of medias at low pH and *Pedicularis bracteosa* appears to be most tolerant of medias at high pH. We are continuing our comparison of the pollen response of species in this genus, and we are designing experiments to investigate variation in stigma and style pH among these species. The flowers of all *Pedicularis* species possess a floral structure (galea) which covers the pistil and anthers and restricts the likelihood of contact between atmospheric precipitation and the sexual parts of the flower. *Penstemon whippleanus* flowers possess a floral tube that results in a similar closed floral morphology. This is in marked contrast to the floral morphologies of *Erythronium* or *Aquilegia*, both of which have fully exposed sexual parts. Since our studies on all of these species have revealed some evidence of sensitivity to exposure to media or rain simulant of pH 3.5 or below, we do not see any evidence at this point to suggest that floral morphology (protected or exposed sexual parts) influences their likely response to the an atmospheric challenge such as acid rain. Our investigation of this question is still continuing, however.

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TABLE ONE

Aquilegia Pollen Growth in Vitro

Meadow Creek Population

Year	Media	Measurement	pH					
			7.5	6.5	5.5	4.5	3.5	2.5
1989	BK-TP	% germination	——	57.9	66.5	69.4	1.4	0.0
		Y tube length (µm)	——	26.5	43.3	40.2	0.0	0.0
		% viability	——	>90	>90	>90	>90	>90
		# grains/well	——	175	176	177	157	130
1990	BK-TP	% germination	38.9	60.8	49.8	53.7	2.9	5.0
		Y tube length (µm)	32.0	38.0	44.0	57.3	34.0	15.0
		% viability	84	86	88	82	86	87
		# grains/well	193	194	196	186	190	189
	BK-S	% germination	32.0	55.2	84.6	19.5	0.0	0.0
		Y tube length (µm)	31.0	80.0	98.0	40.0	0.0	0.0
		% viability	91	90	94	86	90	89
		# grains/well	202	201	194	179	190	183

TABLE TWO

AQUILEGIA POLLEN GROWTH IN VITRO (1990)

ACID-SPRAY FLOWERS VS. AMBIENT-SPRAY FLOWERS

	MEDIA PH					
	7.5	6.5	5.5	4.5	3.5	2.5
ACID - SPRAY FLOWERS						
% GERMINATION	12.1	70.7	75.2	53.0	6.7	11.5
Y TUBE LENGTH (um)	33.3	27.5	56.6	95.0	10.8	34.0
% VIABILITY	89.3	70.7	75.2	89.8	88.0	86.3
# GRAINS/WELL	207	193	196	184	201	188
AMBIENT-SPRAY FLOWERS						
% GERMINATION	50.3	70.0	65.8	74.5	2.8	13.0
Y TUBE LENGTH (um)	56.0	58.3	43.3	93.3	5.8	28.3
% VIABILITY	90.0	92.0	92.0	93.0	87.3	85.7
# GRAINS/WELL	190	198	198	197	190	191

TABLE THREE

AQUILEGIA POLLINATION STUDY (1990)

	TREATMENT	
	ACID-SPRAY	AMBIENT-SPRAY
% Pollinated flowers harvested as fruits	96	89
% Fertile fruit (with ovules)	79	81
Mean # ovules per fruit	113.7	101.1
Mean # maturing seeds per fruit	40.1	35.2
Mean % total ovules/fruit present as maturing seeds	30.3	34.6

TABLE FOUR

Aquilegia caerulea

Date	Mean Stem Height* Acid (n=58)	Control (n=60)	Non-Spray (n=50)
7-12	10.61 [a] (2.69)	11.68 [a] (3.71)	14.09 [b] (4.88)
7-25	13.21 [a] (4.17)	15.63 [b] (4.53)	17.24 [b] (4.51)
8-03	13.44 [a] (5.37)	16.40 [b] (5.28)	19.84 [c] (7.60)
8-13	13.77 [a] (5.54)	16.26 [b] (4.95)	19.71 [c] (7.45)

* Means in centimeters (plus standard deviation)

NOTE: Means with different letters are significantly different at $p < .05$

TABLE FIVE

Aquilegia caerulea

Date	Mean Plant Height* Acid (n=12)	Control (n=10)	Non-Spray (n=10)
7-12	14.92 (2.65)	14.80 (3.76)	18.25 (4.16)
7-25	16.00 (3.36)	16.55 (4.42)	22.95 (3.48)
8-03	17.17 (5.46)	19.30 (5.14)	24.80 (3.97)
8-13	17.92 (7.45)	19.20 (5.49)	23.70 (4.79)

* Means in centimeters (plus standard deviation)

FIGURE ONE

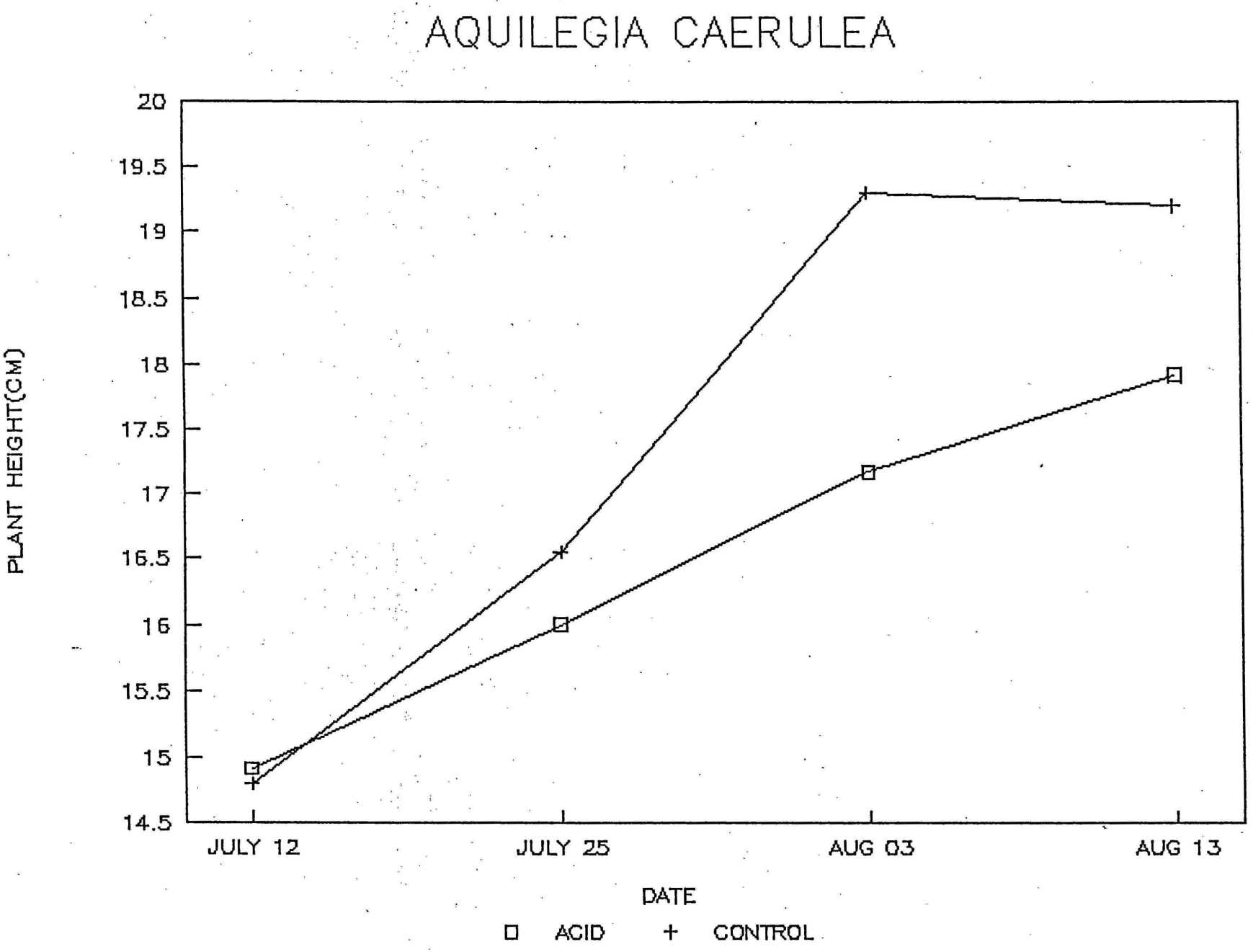

AQUILEGIA CAERULEA
PLANT HEIGHT(CM)
20
19.5
19
18.5
18
17.5
17
16.5
16
15.5
15
14.5
JULY 12
JULY 25
AUG 03
AUG 13
DATE
ACID
CONTROL

TABLE SIX

Aquilegia caerulea

Date	Mean Crown Diameter*		
	Acid (n=12)	Control (n=10)	Non-Spray (n=10)
7-12	16.00 (7.07)	16.10 (5.61)	24.20 (5.53)
7-25	19.75 (8.59)	20.60 (9.18)	33.40 (6.02)
8-03	20.67 (8.51)	23.00 (9.29)	33.70 (8.07)
8-13	21.38 (10.02)	24.00 (11.86)	35.90 (6.59)

* Means in centimeters (plus standard deviation)

FIGURE TWO

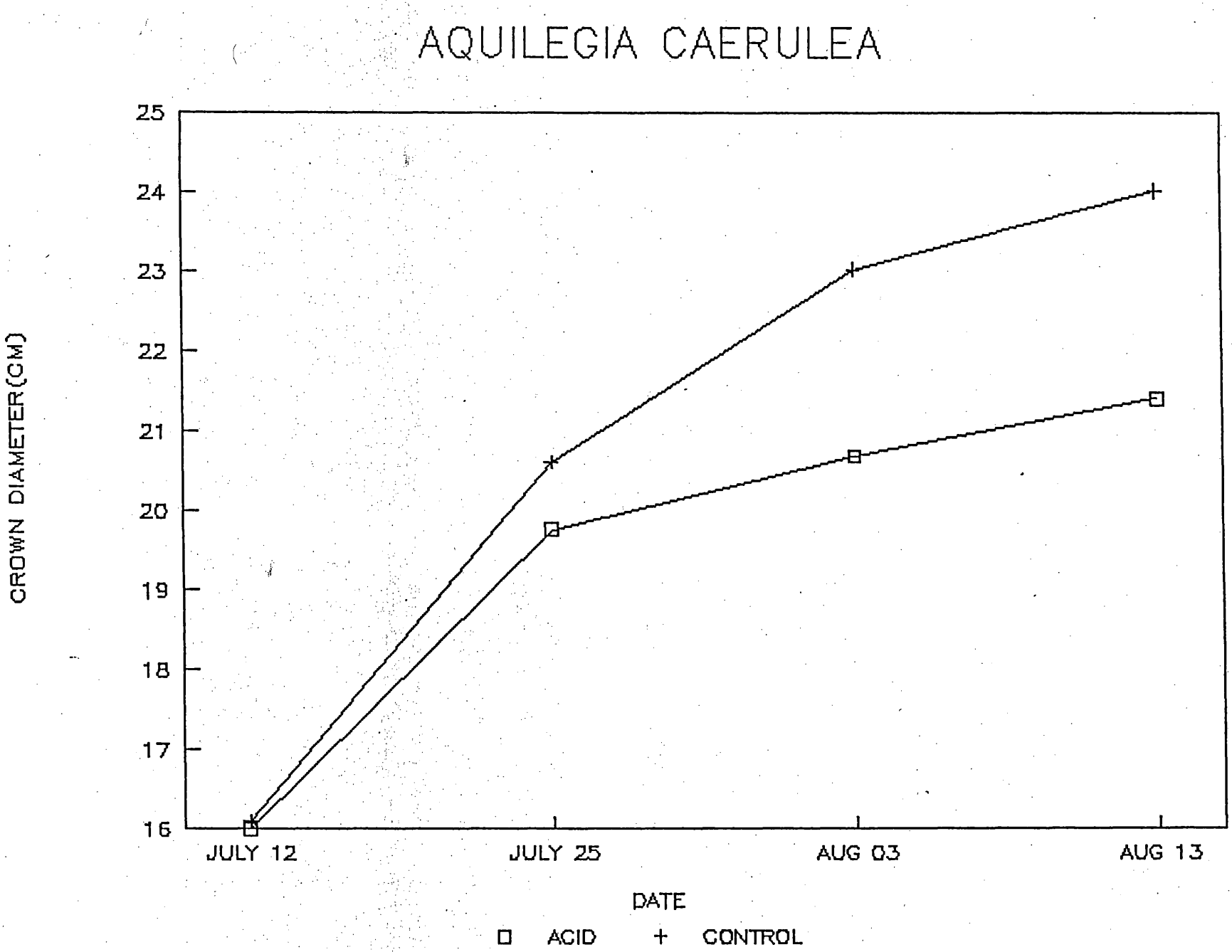

AQUILEGIA CAERULEA
CROWN DIAMETER(CM)
25
24
23
22
21
20
19
18
17
16
JULY 12
JULY 25
AUG 03
AUG 13
DATE
ACID
CONTROL

TABLE SEVEN

Aquilegia caerulea

Date	Total Number of Flowers and Fruits					
	Acid (n=12)		Control (n=12)		Non-Spray (n=10)	
	Flower	Fruit	Flower	Fruit	Flower	Fruit
7-12	1	0	2	0	7	0
7-25	21	0	43	0	91	0
8-03	57	1	80	2	138	1
8-13	40	18	57	19	90	42

TABLE EIGHT

SOIL PH VALUES ASSOCIATED WITH AQUILEGIA GROWTH STUDY

	PLANT TREATMENT		
	ACID-SPRAY	CONTROL-SPRAY	NON-SPRAY
MEASUREMENT DATE			
7/15/90	5.14 (0.29)	5.15 (0.26)	5.19 (0.34)
7/30/90	4.88 (0.42)	5.04 (0.39)	4.97 (0.30)
8/15/90	5.21 (0.32)	5.22 (0.31)	5.28 (0.22)

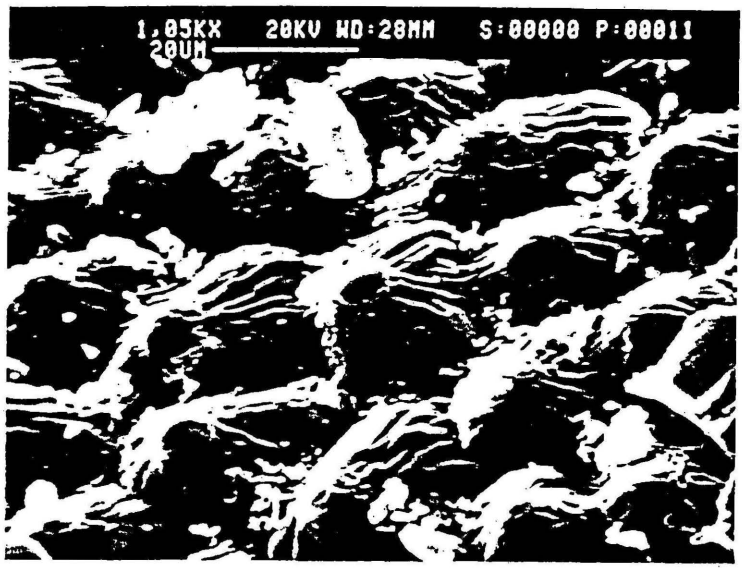
Values represent the mean of samples taken from the vicinity of each experimental plant. Standard deviation is shown in parentheses.

TABLE NINE

LEAF PHYSIOLOGICAL RESPONSE OF PLANTS
IN AQUILEGIA GROWTH STUDY (1990)

	PLANT TREATMENT		
	ACID-SPRAY n = 21	CONTROL-SPRAY n = 19	NO SPRAY n = 15
MEAN CONDUCTANCE (mmol/m/s)	159.6	141.1	100.8
MEAN TRANSPIRATION (mmol/m/s)	3.61	3.67	2.44
MEAN QUANTUM (umol/s)	773.1	1363.5	436.7
MEAN % RELATIVE HUMIDITY	12.9	14.8	13.6
MEAN LEAF TEMPERATURE (degrees C)	15.9	18.3	17.6

Scanning electron micrograph of the abaxial surface of a non-sprayed leaf of Aquilegia caerulea.



Scanning electron micrograph of the abaxial surface of an acid-sprayed leaf of Aquilegia caerulea.

FIGURE THREE

TABLE TEN

Erythronium Pollen Growth in Vitro

Year	Media	Measurement	pH					
			7.5	6.5	5.5	4.5	3.5	2.5
1989	BK-P	% germination	—	69.3	64.5	50.4	29.6	0.0
		Y tube length (μm)	—	—	—	—	—	—
		% viability	—	>90	>90	>90	>90	>90
		# grains/well	—	139	120	172	88	229
1990	BK-S	% germination	85.6	82.0	84.0	68.0	60.5	4.7
		Y tube length (μm)	182	185	147	92	50	33
		% viability	94.6	92.5	83.9	82.1	80.2	80.9
		# grains/well	195	196	202	142	142	176

TABLE ELEVEN

ERYTHRONIUM POLLINATION STUDY (1990)

	TREATMENT	
	ACID-SPRAY	AMBIENT-SPRAY
% Pollinated flowers harvested as fruits	87	89
% Fertile fruit (with seeds)	95	78
Mean # maturing seeds per fruit	42.2	49.2

TABLE TWELVE

ERYTHRONIUM MICROHABITAT STUDY (1990)

SOIL PH MEASUREMENTS

LOCATION 1 : 2" BELOW SNOWPACK

pH READINGS - 4.24, 4.40, 4.16, 4.19, 4.28 MEAN pH = 4.25

LOCATION 2: SURFACE SOIL BELOW SNOWPACK

pH READINGS - 4.06, 4.07, 4.27, 4.05, 4.41 MEAN pH = 4.17

LOCATION 3: SURFACE SOIL AT PERIMETER OF MELTING SNOWPACK

pH READINGS - 4.48, 4.46, 4.06, 4.00, 3.98 MEAN pH = 4.19

LOCATION 4: SURFACE SOIL 4' FROM PERIMETER OF MELTING SNOWPACK

pH READINGS - 4.25, 4.18, 4.08, 4.01, 4.38 MEAN pH = 4.18

TABLE THIRTEEN

PENSTEMON POLLEN GROWTH IN VITRO (1991)

	MEDIA PH						
	8.5	7.5	6.5	5.5	4.5	3.5	2.5
I. OVERALL (n = 45)							
% GERMINATION	77.3	67.7	68.6	56.8	47.2	3.9	0.3
Y TUBE LENGTH (um)	93.5	97.1	87.7	90.7	75.6	37.0	12.4
% VIABILITY	95.3	94.7	94.4	95.5	96.5	95.4	96.7
# GRAINS/WELL	914	826	733	963	1121	869	993
II. MEADOW CREEK POPULATION (n = 20)							
GERMINATION	77.7	68.2	62.1	56.7	55.5	2.7	.02
Y TUBE LENGTH (um)	91.3	85.3	78.1	88.1	79.6	36.3	7.2
III. MOUNTAIN MEADOW POPULATION (n = 19)							
% GERMINATION	79.0	72.8	57.5	61.5	51.9	5.2	0.7
Y TUBE LENGTH (um)	102.4	87.8	93.4	90.0	71.5	26.5	21.6
IV. CENTENNIAL LODGE POPULATION (n = 6)							
% GERMINATION	69.1	49.5	52.2	41.7	26.9	4.5	0.0
Y TUBE LENGTH (um)	90.9	167.6	104.8	100.4	70.7	72.5	0.0

TABLE FOURTEEN

PENSTEMON POLLINATION STUDY (1991)

	TREATMENT	
	ACID-SPRAY	AMBIENT-SPRAY
% Pollinated flowers harvested as fruits	98	96
% Fertile fruit (with seeds)	9	11
Mean # maturing seeds per fruit	14.4	34.0

TABLE FIFTEEN

PENSTEMON WHIPPLEANUS STYLE PH STUDY (1991)

	TREATMENT		
	ACID SPRAY	CONTROL SPRAY	NO SPRAY
POLLINATED STYLES	Y = 5.99 s = 0.16 n = 38	Y = 5.95 s = 0.23 n = 35	Y = 5.97 s = 0.17 n = 26
NON-POLLINATED STYLES	Y = 5.99 s = 0.11 n = 32	Y = 5.95 s = 0.18 n = 35	Y = 5.98 s = 0.09 n = 24

TABLE SIXTEEN

MORPHOLOGICAL MEASUREMENTS OF PENSTEMON WHIPPLEANUS (1991)

LOCATION	MEAN FLOWER LENGTH (cm)	MEAN FLOWER WIDTH (cm)
MEADOW CREEK		
All Flowers n = 46	2.3	0.60
Top Flowers n = 18	2.2	0.50
Middle Flowers - 1 n = 15	2.3	0.60
Middle Flowers - 2 n = 10	2.3	0.70
Basal Flowers n = 3	2.3	0.70
MOUNTAIN MEADOW		
All Flowers n = 71	2.1	0.70
Top Flowers n = 31	2.2	0.70
Middle Flowers - 1 n = 16	2.2	0.70
Middle Flowers - 2 n = 10	2.1	0.70
Basal Flowers n = 14	2.1	0.70

TABLE SEVENTEEN

PEDICULARIS POLLEN GERMINATION IN VITRO (1990)

		MEDIA PH					
SPECIES	MEDIA	7.5	6.5	5.5	4.5	3.5	2.5
P. parryi	BK-TP	33.1 * (249)	35.0 (296)	46.7 (250)	23.6 (180)	0.0 (140)	0.0 (139)
	BK-S	40.0 (191)	58.0 * (193)	68.3 (193)	58.2 (191)	1.6 (191)	0.0 (191)
P. bracteosa	BK-TP	73.2 (519)	57.7 (480)	63.0 * (467)	62.7 (892)	0.1 (535)	0.0 (394)
	BK-S	43.1 (192)	39.9 * (192)	39.9 (191)	1.2 (191)	12.0 (191)	0.0 (191)
P. racemosa	BK-TP	32.5 (212)	26.0 * (199)	50.3 (195)	21.3 (184)	14.5 (148)	9.1 (120)
	BK -S	---	---	---	---	---	---

Values represent % germination at each pH level. Numbers in parentheses indicate the average number of pollen grains per sample. Values marked with * indicates pH where longest pollen tubes were observed.